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Limits of Human Performance**

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**Linking Skeletal Muscle Blood Flow and Metabolism
to the Limits of Human Performance**

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Abstract

Over the last 50 years, Bengt Saltin's contributions to our understanding of physiology of the circulation, the matching of the circulation to muscle metabolism and the underlying mechanisms that set the limits for exercise performance were enormous. His research addressed the key questions in the field using sophisticated experimental methods including field expeditions. From the Dallas Bedrest Study to the 1-leg knee model to the physiology of lifelong training, his prodigious body of work was foundational in the field of exercise physiology and his leadership propelled integrative human physiology into the mainstream of biological sciences.

Key word: Saltin, blood flow, exercise, human, metabolism, performance, muscle.

Introduction

Reflection on the scientific contributions of Bengt Saltin is a study of the history of modern exercise physiology not only by virtue of the breadth of topics in the field to which Bengt contributed, but also through the scientific lineage he was part of, the community of colleagues he collaborated with and the vast number of students he inspired and mentored. I had the fortune of studying with Bengt for many years, beginning at the Copenhagen Muscle Research Centre (CMRC) at its inception in 1994 along with 2 other Canadians (Dave Maclean, Martin Gibala), following other Canadians (Terry Graham, Mike Houston, Burt Taylor, Gabrielle Savard) who had also studied with Bengt years before. The CMRC under Bengt's leadership was a thrilling place and time to study exercise physiology. There were multiple thriving labs (at August Krogh Institute, Panum Institute, Rigshospitalet, Bispebjerg Hospital) led by talented researchers (Galbo, Secher, Richter, Kjaer, Vissing, Pedersen) converging on the central themes of exercise, cardiovascular regulation and muscle metabolism. With ample funding, a large number of students, visiting international scientists, and a vibrant seminar series the CMRC was a 'beehive' of scientific collaboration. Also significant was the series of International Symposia Bengt organized with Inge Holm that brought together virtually all the leading scientists from around the world and which resulted in symposia publications (see Raven, Kjaer, Hellsten 2014; Joyner, Kjaer, Larsen 2015). In that setting, Bengt had a deep understanding of the importance of environment, not only in the study of physiology, but also for students and the collective community of scientists. Commenting on his own path in the field, he related that 'tradition, mentors, the environment he was exposed to and the resulting opportunities he seized were key factors in his scientific life'.

Bengt studied medicine at Karolinska Institute (1956-1959) and was recruited by Ulf von Euler (Nobel Prize for the identification of norepinephrine) to work as a student instructor. Recognizing his talent and interest in exercise and sport, von Euler recommended Bengt pursue graduate study at The Swedish School of Sport and Health Sciences (GIH) under the mentorship of Erik Hohwu Christensen (student of August Krogh) in collaboration with Per Olof Åstrand. Already from 1960-63 Bengt published 5 papers on the topic of oxygen uptake during exercise, and his dissertation entitled “*Aerobic work capacity and circulation at exercise in man*”) was published in 1964 (Saltin 1964). In that and the next 4 years he published prolifically on diverse topics in exercise physiology including the circulation, e.g. *Maximal oxygen uptake in athletes* (Saltin, Åstrand 1967), muscle metabolism, e.g., *Diet, muscle glycogen and physical performance* (Bergström et al. 1967), the effects of environment (heat, altitude), posture, aging and training (Ekblom et al. 1968), and the well-known paper from the Dallas Bedrest Study (Saltin et al. 1968). From 1969-1979 he published 108 papers on similarly diverse topics, and throughout his life, the circulation, exercise training and performance remained one of the central themes of his research. His contributions to our understanding of physiology of the circulation, the matching of the circulation to muscle metabolism and the underlying mechanisms that set the limits for exercise performance were enormous.

The Circulation and Exercise Performance

When asked ‘what are the essential factors in doing good science?’ Bengt’s simple reply was, ‘ask the key questions and employ the best methods’. This approach was evident throughout his life in research. In his 78-page paper ‘*Response to exercise after bed rest and after training*’ published in *Circulation* with Jere Mitchell and colleagues from Dallas (Saltin et al. 1968) the

introduction states, *‘There are no systematic studies of longitudinal design which provide insight into the effects of bed rest and training on submaximal and maximal performance, judged by oxygen uptake, as it relates to respiratory and circulatory factors’*. Already in 1968, sophisticated methods were used to study key questions that are still relevant today. The measurements included oxygen uptake (expired gas measured by Scholander method) cardiac output by dye-dilution, heart volume by radiography, lung diffusion capacity with carbon monoxide, lung volumes by spirometry, residual volume with neon rebreathing, total body water, extracellular fluid and red cell mass by radioisotopes, arteriovenous blood sampling (extensive analytes), muscle biopsies and electron microscopy for capillary density and basement membrane thickness. Diet was controlled in the study which also included high-intensity interval training (HIIT). The premise for the study was indicative of Bengt’s integrative approach to physiology that incorporated questions common to both the patient in the clinical setting and the elite athlete, *‘The capacity to adapt not only to short bursts of exercise, but also to longer periods which are frequently repeated over longer periods of time is fundamental to survival. It involves both the capacity to return to normal activity after prolonged immobilization and, in the case of the athlete, to train for competitive events.....* The paper had a significant clinical impact. At that time the incidence of myocardial infarction was at its peak in US history, and subsequent to the publication of the paper, cardiac rehabilitation post-infarction changed from bedrest to exercise as a mainstream approach. For the athlete, the study showed the relative contributions of cardiac output (central) and muscle O₂ extraction (peripheral) in the training and detraining response. The study illustrated the profound decrement in heart volume, cardiac output and oxygen uptake with bedrest (inactivity) and the combined central and peripheral improvements with training. Bengt revisited this theme from many perspectives with incrementing experimental

sophistication to gain understanding of mechanisms –a scientific approach that was characteristic of Krogh and emphasized by his mentor Christensen as exemplified in a closing statement at the Biochemistry of Exercise meeting in Magglingen, 1974 *“I envy you! My generation of researchers have described several of the acute and chronic responses to exercise. You, have the tools, the methods and the knowledge to unravel the mechanisms by which these processes are regulated. Remember, not until we understand these regulatory mechanisms, has our research goal been reached”*.

Leading up to the publication on the 1-leg knee extension model (Andersen & Saltin 1985), Bengt conducted several studies on the training response to 1-leg exercise (cycling) comparing sprint and endurance training and immobilization (Saltin et al. 1976; Saltin, 1977), showing that the cardiovascular and muscle metabolic responses are inter-dependent, specific to the training stimulus and to the muscles engaged in training. This topic was also of interest to Christensen based on an earlier publication (Christensen, 1931) *“How is the heart able to sense whether the contracting muscles are trained or not?”* Bengt addressed this question in some of his last publications showing that with cardiac pacing the elevated cardiac output during exercise is regulated by the increase in skeletal muscle blood flow and venous return to the heart, whereas the increase in heart rate appears to be secondary (Mortensen et al. 2013). Combining cardiac pacing with a 1-leg training model, the lower cardio-ventilatory response to exercise was linked to a reduced signaling from trained skeletal muscle while a lower cardiac afterload increased stroke volume (Bada et al. 2012).

The 1-leg knee extension model, first published as an abstract in 1982 (Andersen 1982) was a natural extension of his work in the 70's. Ongoing experiments at that time provided context for the discussion of factors limiting exercise performance and the cardiovascular and

muscle adaptations to training covered in two comprehensive reviews in 1983 (Saltin & Gollnick 1983; Saltin & Blomqvist 1983). Much of our basic knowledge on the cardiovascular adaptations to training in humans was outlined in his review with Blomqvist including the important role of stroke volume, cardiac output and O_2 delivery for VO_2 max, and the role of the autonomic nervous system in the control of vascular conductance. The reduction in sympathetic vasoconstriction with training was suggested to be a local effect (sympatholytic) in muscle which also had central effects on afterload allowing maximal cardiac to be increased. The expansion of muscle capillary volume was considered the primary mechanism for increased muscle O_2 diffusion capacity -termed the 'transfer coefficient for O_2 ' (ml/min/mmHg) at that time. Changes in mitochondrial capacity were considered to have no direct causal link to maximal oxygen uptake, but rather the upregulation of mitochondrial volume with training contributed to endurance performance or time to exhaustion at submaximal loads. The role of mitochondria for endurance performance stemmed from regulatory changes in fat oxidation. These patterns and underlying mechanisms were again followed up and confirmed in later studies combining measures of O_2 delivery and mitochondrial respiratory capacity (Boushel et al. 2014; 2015).

With the publication of the knee extension model (Andersen & Saltin 1985) Bengt expanded on the central hemodynamic and peripheral (vascular or metabolic) contributions to maximal oxygen uptake (Saltin 1985). He argued that the heart (cardiac output) was the limiting factor since the quadriceps muscle could accommodate a 2-fold higher flow rate (>250 ml/min/100g) and achieve a 2-fold higher VO_2 (350 ml O_2 /min/100g) at peak 1-leg exercise compared to 2-leg cycling. It was concluded that in whole body exercise, *'the capacity of the muscles to receive flow exceeds by a factor of 2-3 the capacity of the heart to supply the flow'*. Therefore, at peak whole body exercise, vasoconstrictor tone directed to feed arterioles

curtailed maximal blood flow to muscle. The high flow rate to the isolated quadriceps muscles was accompanied by a lower O_2 extraction of 70-75% compared to 80-85% with 2-leg cycling, which was related to the mean transit time of erythrocytes through capillaries. Since the muscle capillaries and oxidative apparatus could accommodate these higher rates (flow, VO_2), he proposed that capillary volume expansion with training served to increase mean transit time to allow for a higher diffusion of oxygen, rather than to accommodate a higher flow. Bengt's work and thinking on the role and limits of blood flow for muscle oxygen uptake and exercise performance stemming from this experimental work in the 80's remain a foundation of our knowledge today.

Regulation of Muscle Blood Flow

In the early years of the Copenhagen Muscle Research Centre (CMRC), a main focus in the cardiovascular area was the study of local factors regulating muscle blood flow during exercise. A long standing question Bengt had was what vasodilator (mechanisms) linked oxygen delivery to muscle metabolic demand and what metabolic signal evoked the vasodilator response to match oxidative demand. Some of the first work examined the role of adenosine showing that adenosine increases in muscle interstitium with exercise intensity and the magnitude of muscle blood flow (Hellsten et al. 1998) and a similar response of leg blood flow in response to arterial adenosine infusion with that of voluntary exercise including the kinetics of the response (Saltin et al. 1998; Rådegran and Calbet 2001). The effect of nitric oxide (NO) for regulating muscle blood flow was found to be lower than adenosine when NO was induced by infusion of acetylcholine (Rådegran & Saltin 1999). Pharmacological studies examined the blood flow response to exercise with inhibition of adenosine and nitric oxide with aminophylline and N^G -Monomethyl-L-arginine monoacetate (L-NMMA) respectively, while others had conducted

similar inhibition experiments examining prostaglandins (PG) (Shoemaker et al. 1996). At that time it was only the inhibition of adenosine that produced a modest reduction in limb blood flow during exercise (Saltin et al. 1998). In a subsequent study that applied combined pharmacological inhibition of nitric oxide and prostaglandins (Boushel et al. 2002) blood flow was substantially reduced leading to the concept of redundancy in vasodilator responses, and which explained the lack of effect of single vasodilator inhibition on blood flow. Follow-up studies demonstrated interactions amongst adenosine, prostaglandins and nitric oxide. Not only did NO and PG release by the vascular independently elicit vasodilation but they were also a part of the adenosine-mediated vasodilator response to exercise (Nyberg et al. 2010). The combination inhibitor studies involving nitric oxide and PG also revealed the dual role of nitric oxide as a regulator of exercise hyperemia and a modulator of mitochondrial respiration resulting in greater mitochondrial efficiency (Hillig et al. 2003; Mortensen et al. 2007; Boushel et al. 2012). Studies ensued on ATP as it is a powerful dilator and its release from the red blood cell is enhanced as the saturation of hemoglobin decreases with exercise intensity when oxygen extraction increases (Gonzalez-Alonso et al. 2008; Ellsworth 2009). ATP was considered a potential mediator of an O₂ sensing mechanism based on Bengt's earlier findings that the regulation of cardiac output, leg blood flow and O₂ delivery to muscle was dependent on signaling linked to arterial oxygen 'content' (CaO₂) of blood rather than the O₂ pressure (PaO₂) (Koskolou et al. 1997; Roach et al. 1999). Similar to adenosine, a portion of vasodilation induced by ATP was derived from PG and NO release (Mortensen et al. 2009) in addition to purinergic receptor (P2Y) binding on luminal endothelial cells causing hypopolarization via inwardly rectifying potassium channels with downstream smooth muscle cell relaxation (Crecelius et al 2012). ATP was also released from skeletal muscle and shown to increase in the interstitium

where P2Y receptors also exist and in contrast to adenosine was shown to inhibit the effect of sympathetically released norepinephrine acting on α -receptors (Hellsten et al 2012).

The sympatholytic portion of ATP-induced vasodilation was explored in several papers. Functional sympatholysis and muscle blood flow were found to be impaired in elderly sedentary compared to young men, and in young subjects after leg immobilization, whereas regular physical activity prevented age and immobility-related impairments (Saltin, Mortensen 2012). Training increased skeletal muscle P2Y2 receptor content located in the endothelium of microvessels and smooth muscle cells, and P2X1 receptors located in the endothelium and the sarcolemma (Mortensen et al. 2009). The training effect was seen in normotensive and hypertensive individuals (Mortensen et al. 2014). To further explore the mechanisms of vasodilation by purinergic receptors and to determine the relative potency of nucleotide/nucleoside vasodilator pathways, the vasodilation and blood flow response to adenosine, ATP and UTP (uridine-triphosphate) as well as expression/distribution of P2Y(2) and P2X(1) receptors in skeletal muscles were examined in healthy and diabetic subjects (Thaning et al. 2011). The study showed hierarchical vasodilatory potency of the nucleoside/nucleosides (UTP > ATP > ADO) and a 50% reduction in purinergic vasodilation in diabetics.

In a talk Bengt gave in 2013 he summarized the work done in the field on the local mechanisms of muscle blood flow regulation during exercise. Despite some of the experimental limitations such as the lack of available and/or specific pharmacological inhibitors (e.g. purinergic), the magnitude of exercise hyperemia could be reasonably accounted (up to ~85-90%) for by the multiple redundant vasodilator substances studied to date, when also including the effect of the muscle pump. That being said, he also believed that much more work was needed to understand the precise mechanisms and interactions of the various regulators.

In Bengt's last studies on a unique group of men who maintained regular exercise training for ~40 years, it was shown that functional sympatholysis and the vasodilator response to arterial ATP infusion remained intact (Mortensen et al. 2012). This was part of a larger study examining numerous cardiovascular responses to exercise including the finding that the lifelong training prevented age-related increases in endothelin-1 (Nyberg et al. 2013). Atrio-ventricular axis shortening in the heart was also preserved in lifelong trained (similar to young individuals) and this effect accounted for the large (higher) maximal cardiac output response during dynamic exercise compared to untrained (Steding-Ehrenborg et al. 2015). Additional unique findings on the convective and diffusive components of muscle VO_2 and muscle mitochondrial capacity presented at the Saltin CSEP Symposium 2015 await publication. Many of these findings, especially on 'lifelong trained' provided for Bengt, the physician physiologist a strong basis for his publications on 'Exercise as Medicine' (Pedersen, Saltin 2015).

Field Studies.

During the CMRC years Bengt led 2 major field expeditions that included the study of cardiovascular regulation at high altitude (The Chacaltaya Expedition, 5260m, Bolivia 1998, **Figure 1**) and the cardiovascular, metabolic adaptations to training in the arctic (Greenland Expedition, Qaanaaq 2004). As a background, his mentor Christensen joined the 1935 high altitude expedition in the Andes of Chile, where two striking findings were described: the lactate paradox (Edwards 1936), and the low maximal heart rate (bradycardia) at altitude (Christensen and Forbes 1937). The mechanisms underlying these responses remained unsolved for over 65 years and Bengt was eager to investigate the mechanisms. Both questions were answered in the Chacaltaya study: the lactate paradox at altitude was challenged (Lundby et al. 2000; van Hall et al. 2001), and the bradycardia at altitude was attributed to enhanced parasympathetic tone to the

heart (Boushel et al. 2001). The expedition resulted in 14 papers, 7 of which focused on cardiovascular regulation (e.g. Calbet et al. 2009). Among the many findings on the circulation were: 1) a profound (centrally-mediated) reduction in peak cardiac output, leg blood flow and power output at maximal exercise, 2) the attenuated heart rate response (bradycardia) at maximal exercise had no impact on peak cardiac output or leg blood flow, 3) both the reduction in plasma volume and hemoconcentration at altitude had no impact on peak cardiac output, 4) peak muscle blood flow and VO_2 during 1-leg KE were preserved at altitude.

Bengt had a special interest in the physiology of cross-country skiing. He had worked with Swedish Olympic skiers and conducted seminal studies on the circulatory and metabolic responses in arms and legs during skiing with HC Holmberg, Jose Calbet and colleagues in Stockholm (Calbet et al. 2004; 2005; Holmberg 2015). In several ski expedition studies spanning 30 years, the mechanisms underlying the dissociation between training-induced improvements in muscle oxidative capacity and peak VO_2 (lack thereof) remained unexplained. In the Greenland study, the effect of prolonged low-intensity training (42 days skiing) on systemic and local O_2 delivery, muscle O_2 diffusion and mitochondrial function in arms and legs were examined. Consistent with the previous field studies, peak pulmonary VO_2 was unchanged after ski-training yet arm VO_2 and power output were increased along with a higher arm blood flow and expanded muscle capillary volume (Boushel et al 2014). Muscle O_2 diffusion capacity and O_2 extraction were enhanced at a similar mean capillary transit time and P_{50} of hemoglobin, whereas mitochondrial O_2 flux capacity was unchanged. Thus, significant local improvements in peak muscle VO_2 occur with low intensity training, which are not detectable at the systemic level. In addition to the physiological findings, this also emphasized the importance of the experimental methods for uncovering mechanisms. The increase in peak arm VO_2 was due to increased

convective O₂ delivery to muscle combined with a larger capillary-muscle surface area that enhanced diffusional O₂ conductance. There was an increase in mitochondrial fat oxidation capacity with no change in maximal (mitochondrial) respiratory capacity, and therefore a larger fraction of mitochondrial excess capacity was utilized at peak exercise after training. For leg exercise, there was also no change in pulmonary VO₂, and unlike the arms there was no increase in peak leg VO₂. Interestingly, leg VO₂ was maintained despite a 25% reduction in mitochondrial respiratory capacity, demonstrating both excess capacity and changes in mitochondrial efficiency with training (Boushel et al. 2015). The study also followed up on the earlier studies in Stockholm in elite skiers by examining the circulatory responses during arm and leg exercise with training (Holmberg 2015; Calbet et al. 2015). Aligning with his thinking from the 1980's, the autonomic nervous system played a key role in partitioning cardiac output to arms and legs as a function of relative intensity, resulting in limb-specific vascular conductance that allows for a similar peak VO₂ per kg of muscle mass in both extremities.

Conclusion

Bengt Saltin was a remarkable scientist, leader, and pioneer in the field, and a friend to many. He published prolifically in his career, demonstrating enormous breadth of knowledge in human physiology. He had a brilliant and curious mind, his love of the field was contagious, and he made those around him feel that they were part of something big. His list of publications would be significantly longer had he not preferred to allow those emerging in the field to be recognized independently. He was very generous through providing opportunities and guidance to young scientists. More than 15 of the students from the CMRC became full professors. Jerry Dempsey, his long-time friend and colleague considered Bengt 'as the scientist most responsible in the

world for the remarkable development of the field of integrative physiology in the last 50 years and for his role in securing the legitimacy of our science' (Editorial, 2015). Amongst his many awards, the August Krogh Prize was a special one as Bengt was closely connected to Krogh's scientific lineage and Bengt in his era rigorously continued and exemplified that legacy. In the book by Bodil Schmidt-Nielsen, 'August and Marie Krogh- Lives in Science', Eugene Landis wrote of Krogh *'it is rare enough that a scientist sheds light on an area which nature has kept secret and then during his lifetime sees that area illuminate in every corner of physiology and medicine..... That meanwhile he should develop in addition a large school of devoted students who loved the man even more, if possible, than they venerated the scientist, spells the highest form of genius'*.

The same can be said about Bengt.

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Figure 1.

Laboratory experiment on cardiovascular regulation during exercise at high altitude from the 1998 Chacaltaya Expedition, Bolivia. The laboratory was housed in the Cosmic Ray Laboratory on Mount Chacaltaya, at 5260 m. Pictured left to right: Jose Calbet, MonaTykgaard Mortensen (study volunteer), Bengt, Robert Boushel, Morten Hansen (study volunteer).

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